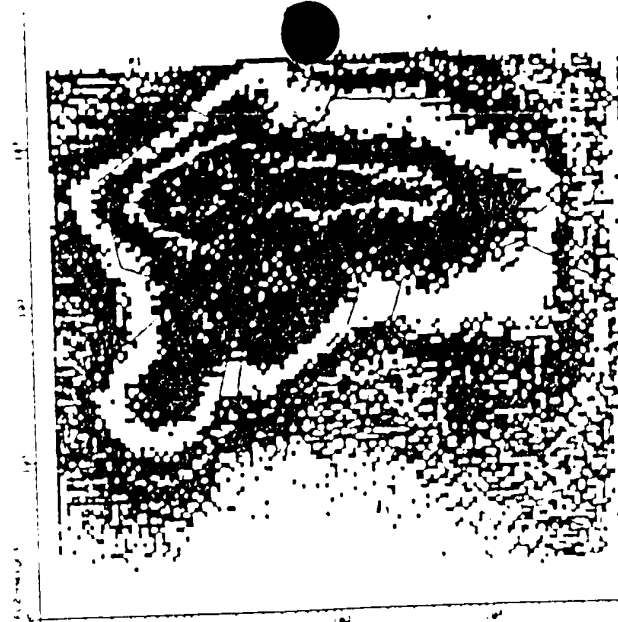


SCANNED, # 22

Figure 1

A

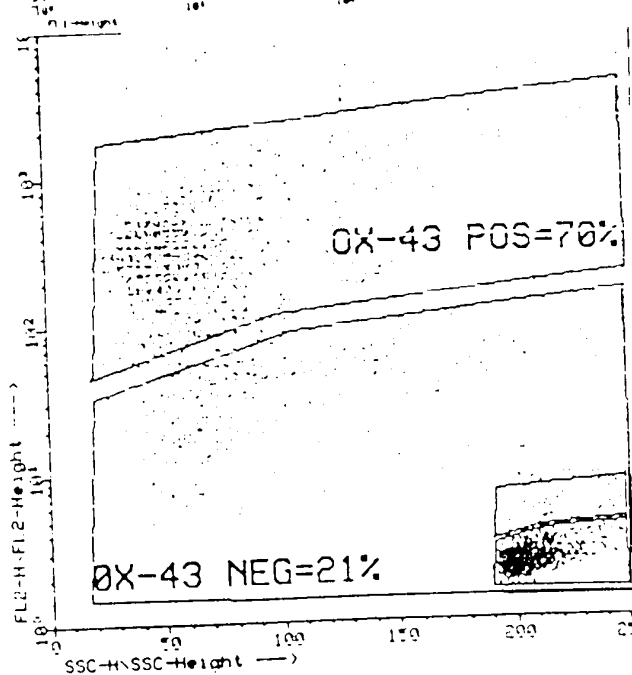


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B



C

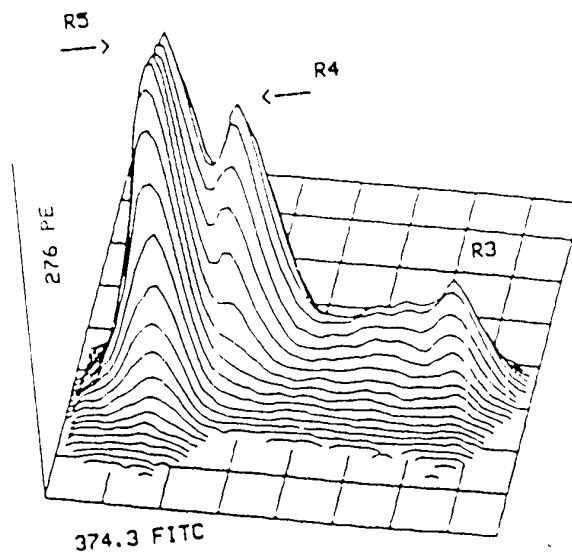


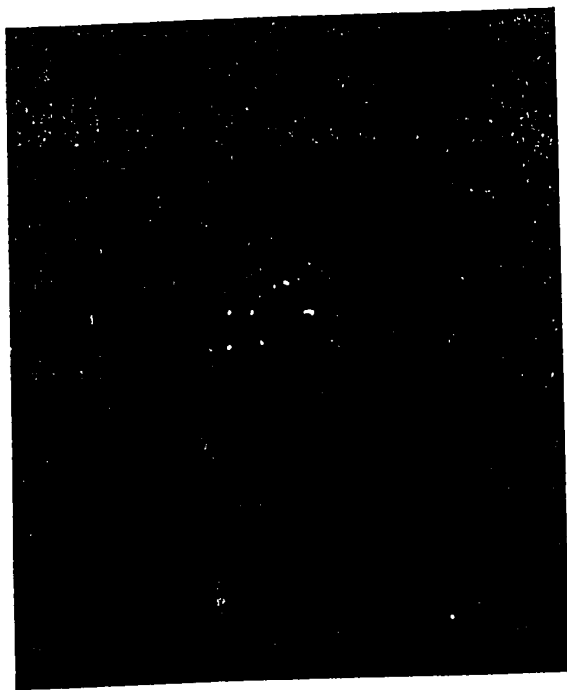
Figure 2

OX-43⁻
OX-43⁺

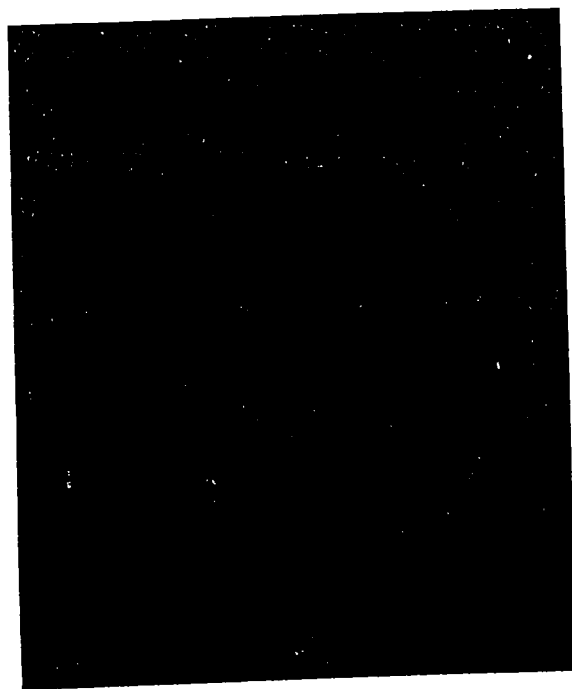


A

SCANNED # 20



B



C

Figure 3

R3 R4 R5

Albumin



Serglycin

SCANNED # 22

SCANNED # 20

Figure 4

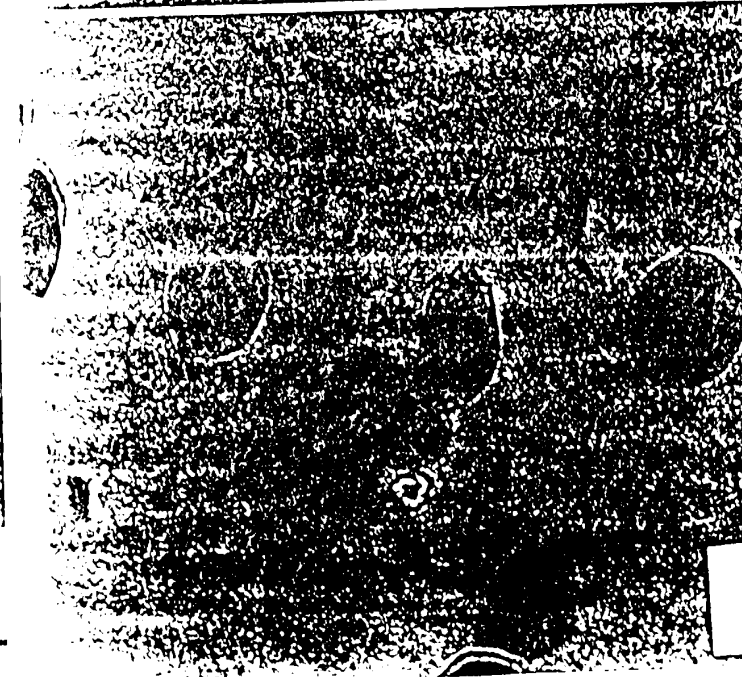
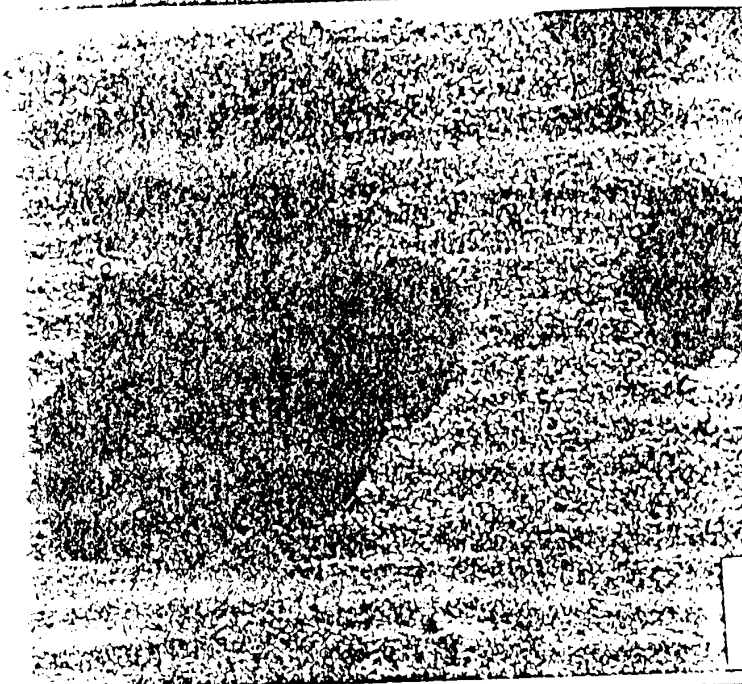
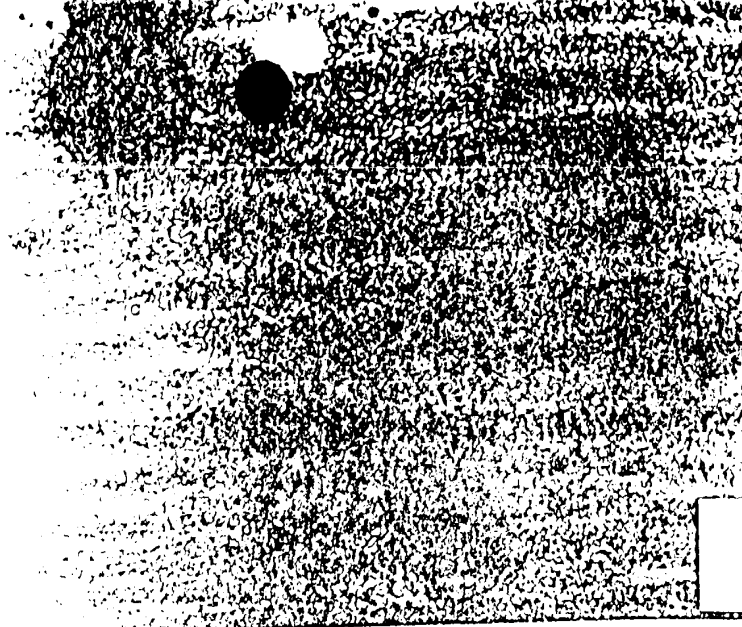
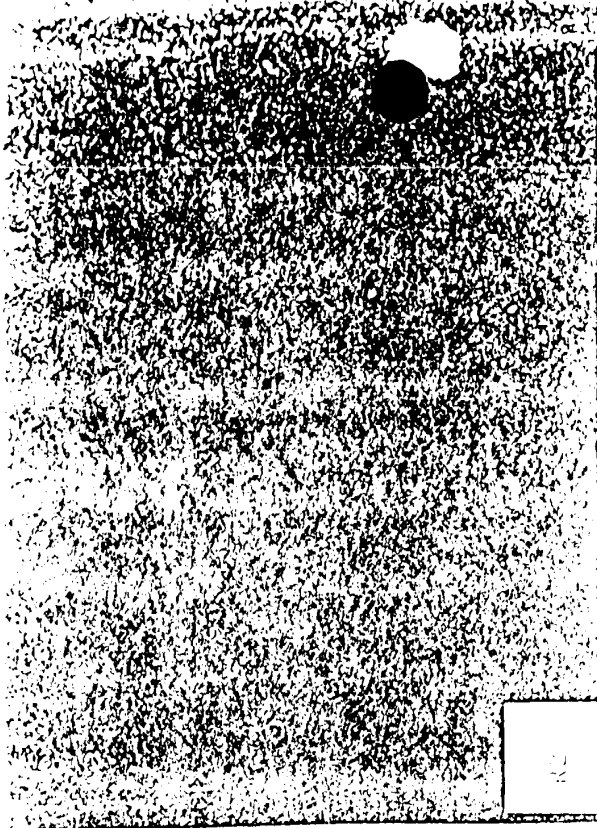
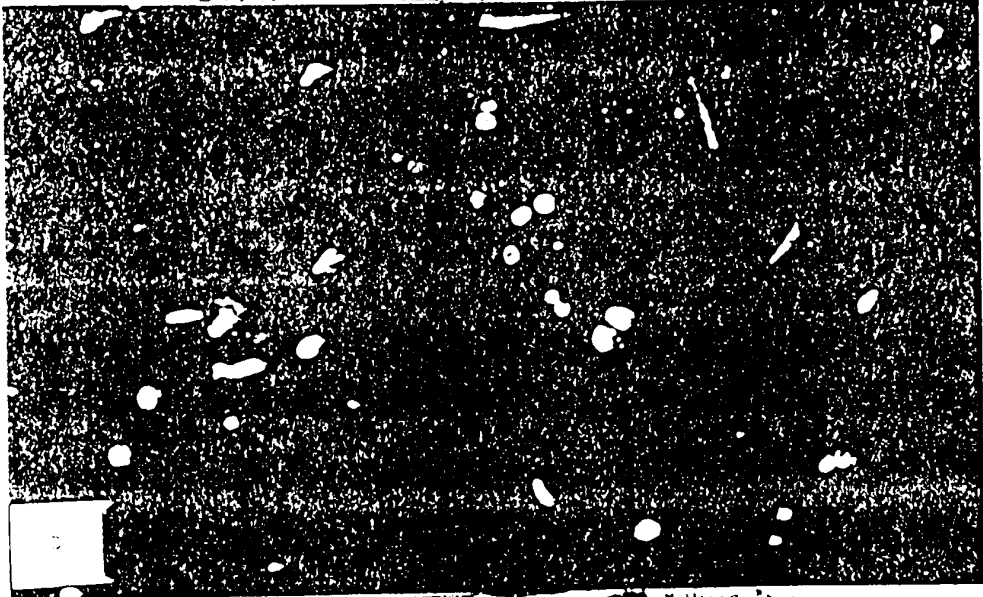
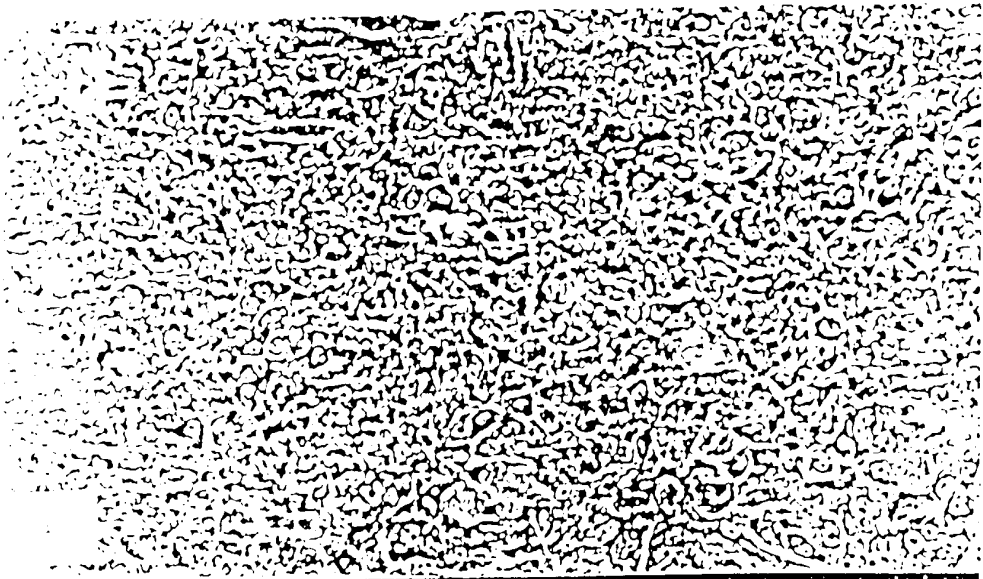


Figure 5



SCANNED # 20

Flow Diagram of Hepatoblast Enrichment

Livers (8-9 mgs)

↓ Dispersion with EGTA and then collagenase

Single Cell Suspension Preparation: Collagenase,
EGTA, 4°C

↓ 10^7 cells/8 mgs liver

↓ $3.2 \pm 1.3\%$ are ALB⁺

↓ $2.5 \pm 0.7\%$ are AFP⁺

↓ $87.9 \pm 2.5\%$ are OX43/44⁺

Panning

Red Blood Cell Panning (2X)

↓ $29 \pm 5\%$ of cells remain

↓ $9.5 \pm 1.2\%$ are ALB⁺

↓ $9.8 \pm 0.9\%$ are AFP⁺

↓ $80.4 \pm 3.9\%$ are OX43/OX44⁺

OX-43/OX-44 Panning (myeloid and endothelial cells)

↓ $16 \pm 4\%$ of cells remain

↓ $14.8 \pm 3.6\%$ are ALB⁺

↓ $14.9 \pm 2.5\%$ are AFP⁺

↓ $69 \pm 10\%$ are OX43/OX44⁺

Fluorescence Activated Cell Sorting

Negatively Sort for Contaminant Cell Populations:

OX-43 (CD 11b)/OX-44 (CD37)⁺ Cells = precursors and mature forms of hemopoietic cells
(myeloid, erythroid) and endothelial cells

Of remaining cells (OX-43⁻ + OX-44⁻ cells), sort for cells varying in OC.3
expression and granularity:

OX-43/(CD 11b)/OX-44 (CD37)⁺ Cells = mostly hepatic precursors, some residual hemopoietic
cell contaminants, stromal cells

OC.3⁺, granular cells = committed bile duct precursors (AFP⁺, ALB⁻)

OC.3⁻, granular cells = committed hepatocyte precursors (AFP⁺, ALB⁺⁺⁺)

OC.3⁺ agranular cells = early hepatoblasts (AFP⁺⁺⁺, albumin⁺ and CK 19⁻)

SCANNED, # 20

Figure 7

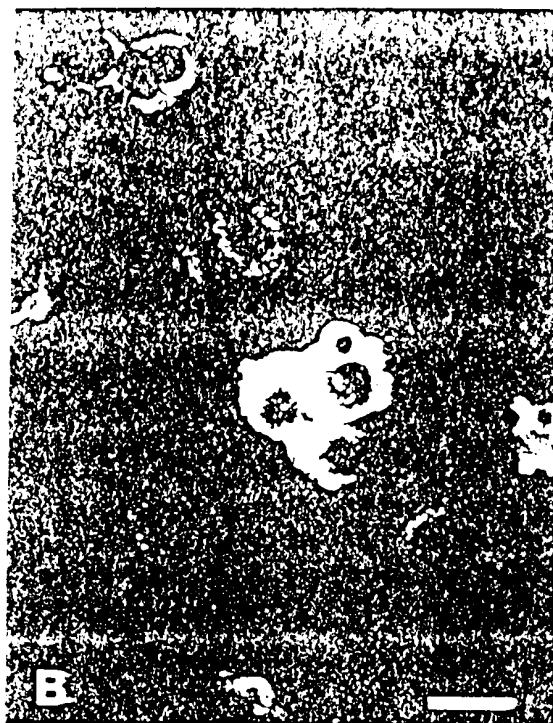
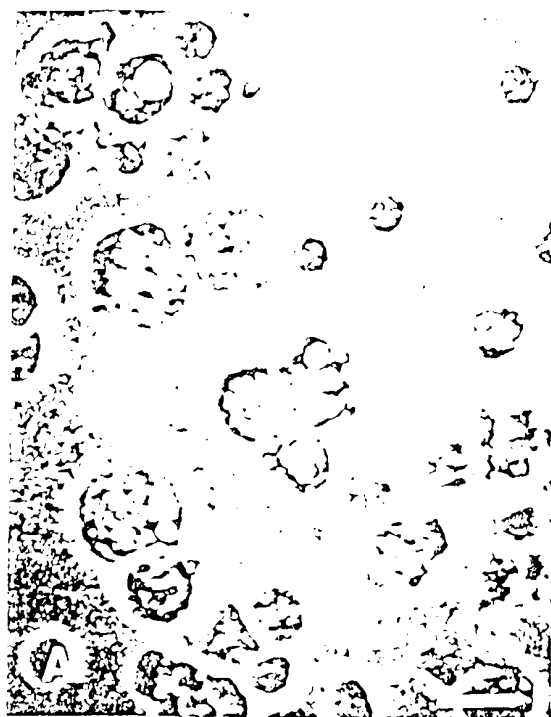


Figure 8

SCANNED # 22

18S

AFP



Original suspension

Panned cells

18S

Alb

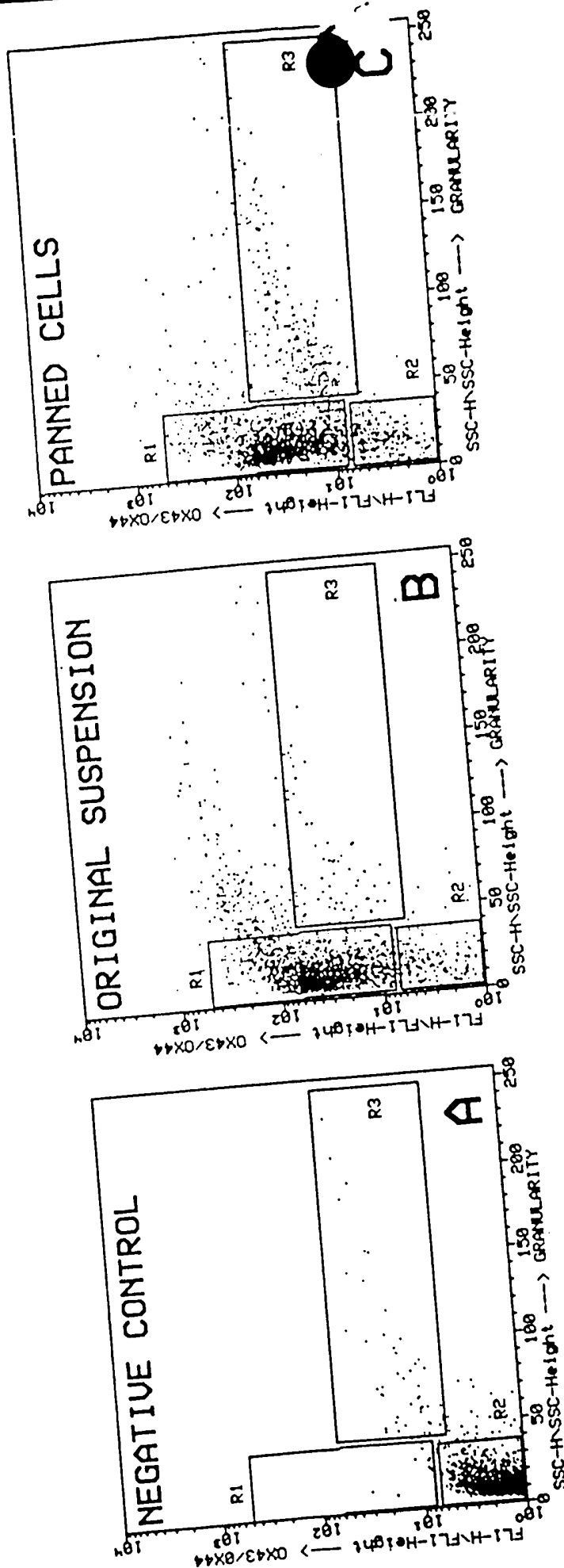


Original suspension

Panned cells

SCANNED, # 80

Figure 9



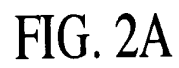
OX-43⁺

FIG. 2B

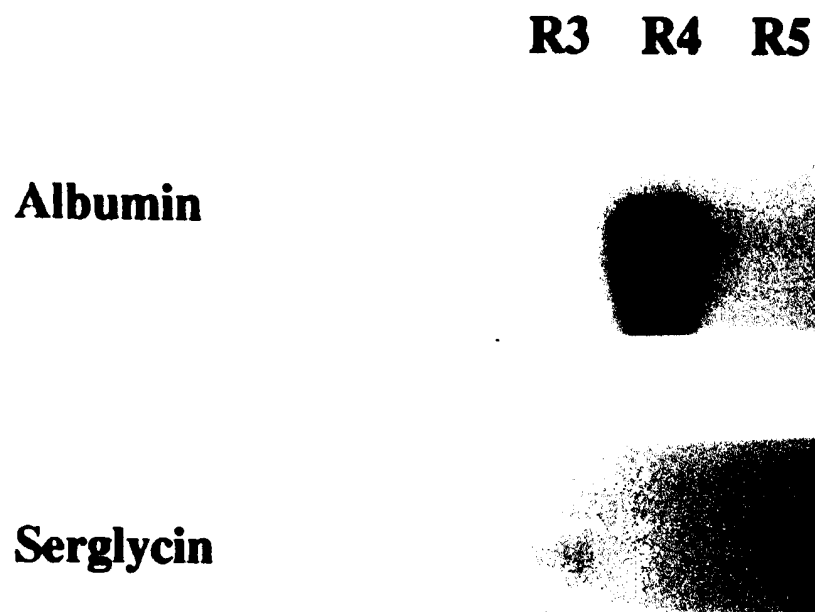


FIG. 3

The image is a composite of five panels, each labeled in the bottom-left corner. Panel R1 is a grayscale micrograph showing a cell with a prominent nucleus. Panel R2 is a solid black square. Panel R3 is a solid black square. Panel R4 is a solid black square. Panel R5 is a color micrograph showing a flower-like structure with five dark petals and a lighter center.

374.3

FIG. 4

8/11

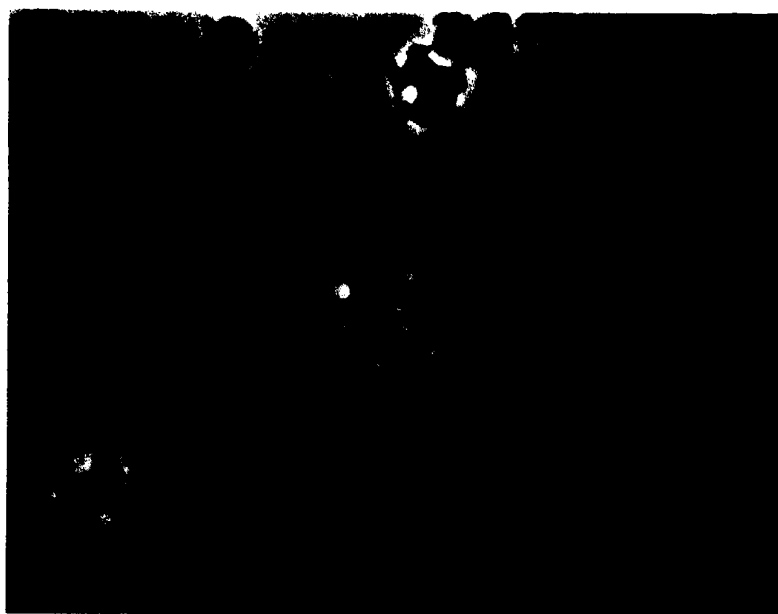


FIG. 7A

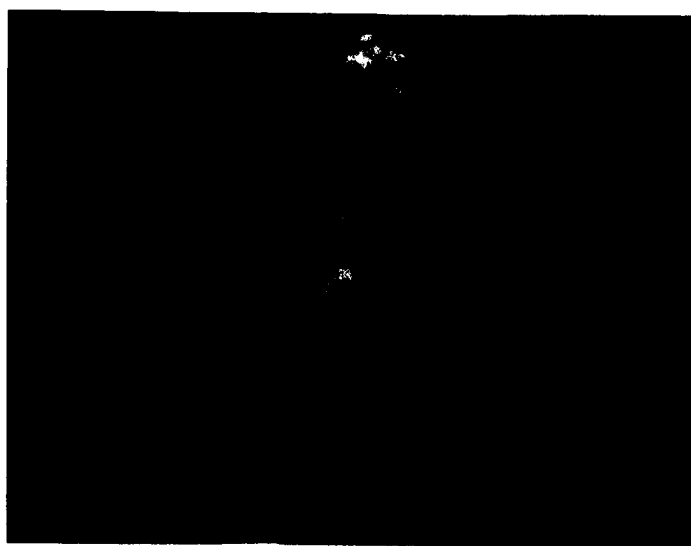


FIG. 7B

9/11

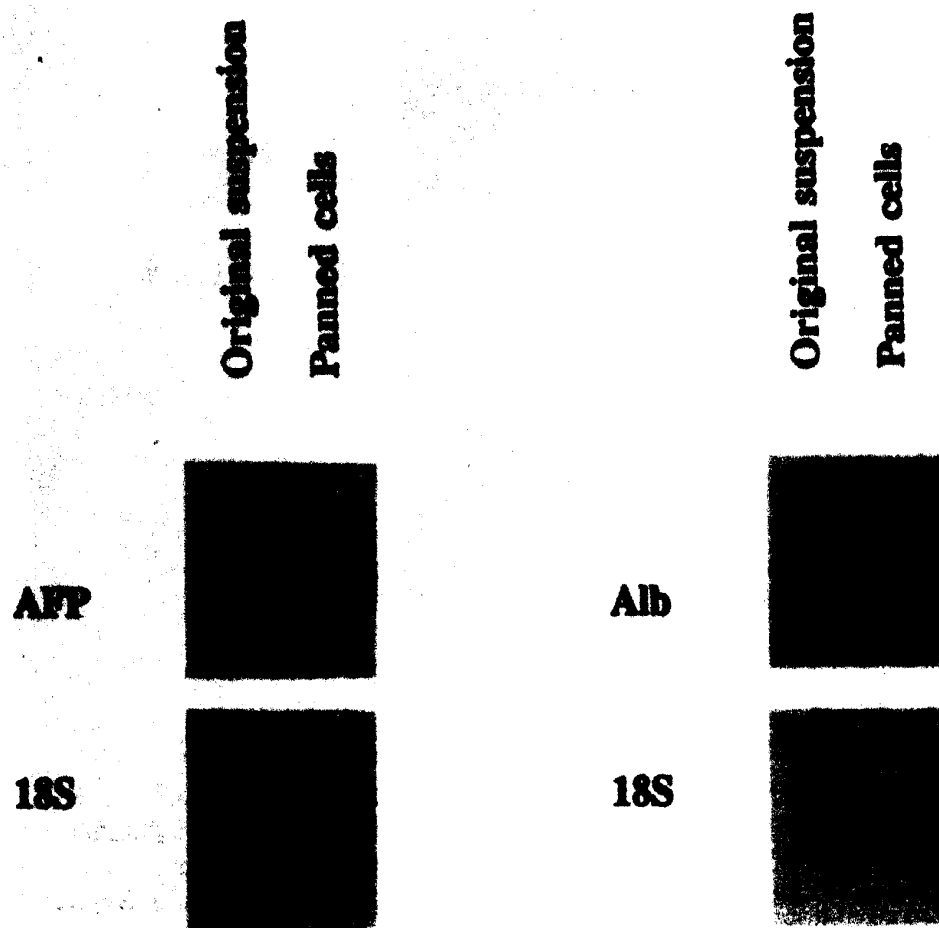


FIG. 8

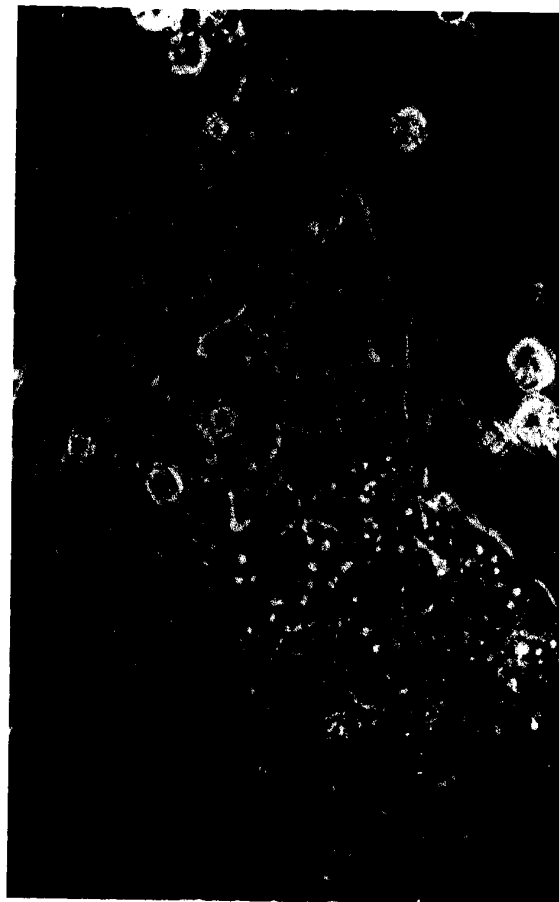


FIG. 10

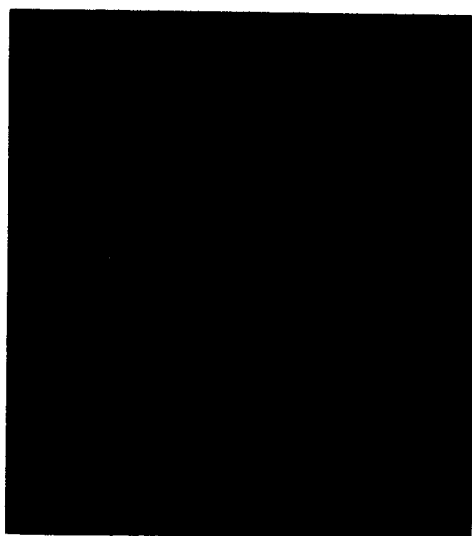


FIG. 11

105050-4322850

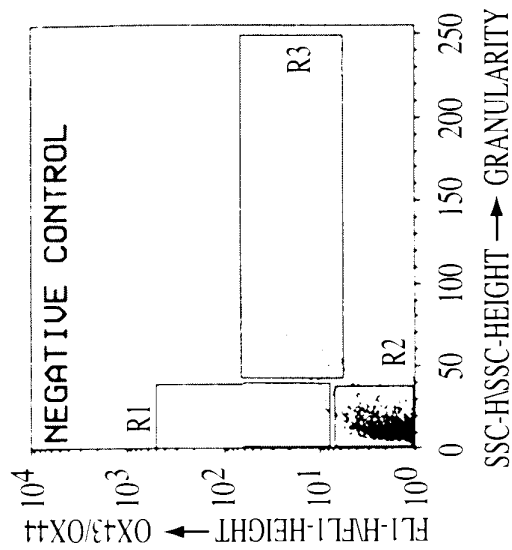


FIG. 9A

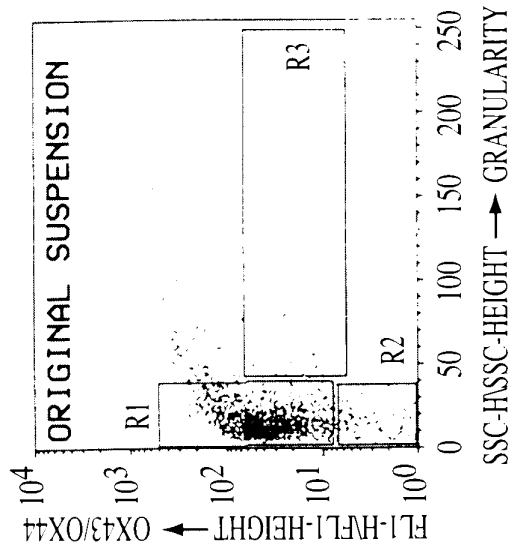


FIG. 9B

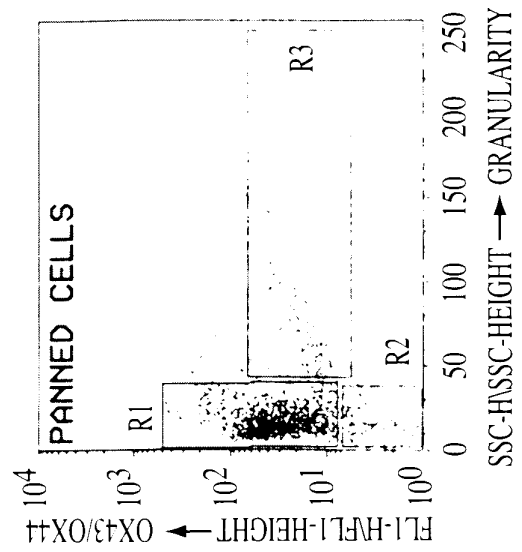


FIG. 9C

6/11



FIG. 5B

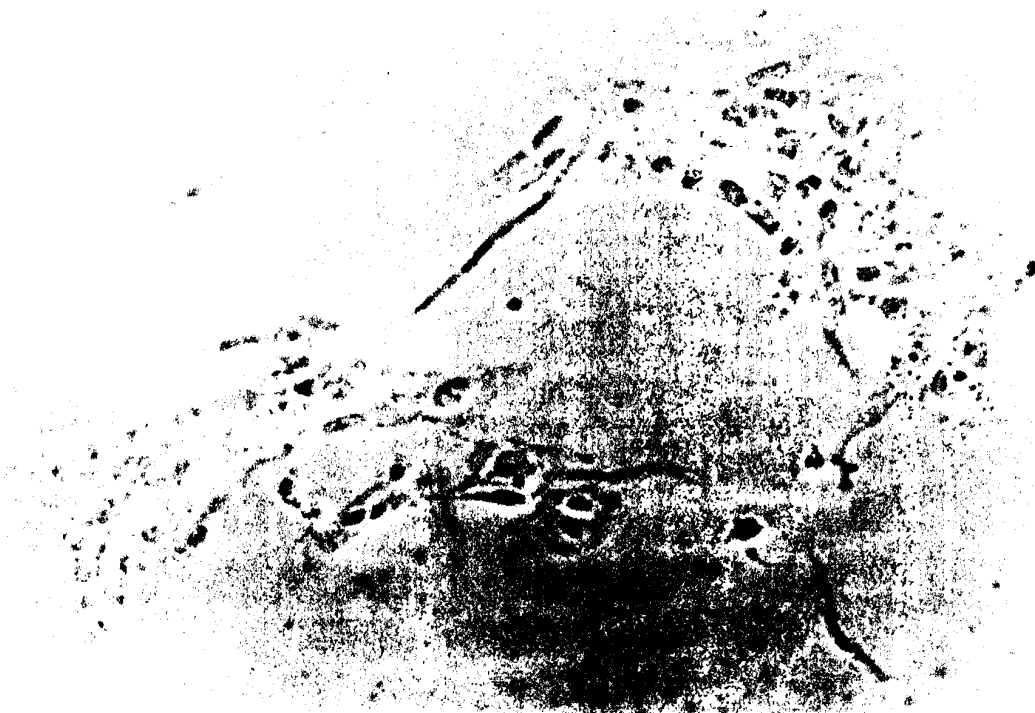


FIG. 5C

7/11

FLOW DIAGRAM OF HEPATOBLAST ENRICHMENT

LIVERS (8-9 mgs)

↓ DIRSPERSION WITH EGTA AND THEN COLLAGENASE

SINGLE CELL SUSPENSION PREPARATION: COLLAGENASE,
EGTA, 4° C

↓ 10^7 CELLS/8 mgs LIVER

↓ 3.2 ± 1.3 % ARE ALB⁺

↓ 2.5 ± 0.7 % ARE AFP⁺

↓ 87.9 ± 2.5 % ARE OX43/44⁺

PANNING

RED BLOOD CELL PANNING (2X)

↓ 29 ± 5 % OF CELLS REMAIN

↓ 9.5 ± 1.2 % ARE ALB⁺

↓ 9.8 ± 0.9 % ARE AFP⁺

↓ 80.4 ± 3.9 % ARE OX43/OX44⁺

OX-43/OX-44 PANNING (MYELOID AND ENDOTHELIAL CELLS)

↓ 16 ± 4 % OF CELLS REMAIN

↓ 14.8 ± 3.6 % ARE ALB⁺

↓ 14.9 ± 2.5 % ARE AFP⁺

↓ 69 ± 10 % ARE OX43/OX44⁺

FLUORESCENCE ACTIVATED CELL SORTING

NEGATIVELY SORT FOR CONTAMINANT CELL POPULATIONS:

OX-43(CD)/OX-44(CD37)⁺ CELLS = PRECURSORS AND MATURE FORMS OF HEMOPOIETIC CELLS
(MYELOID, ERYTHROID) AND ENDOTHELIAL CELLS

OF REMAINING CELLS (OX-43⁻ + OX-44⁻ CELLS), SORT FOR CELLS VARYING IN OC.3
EXPRESSION AND GRANULARITY:

OX-43(CD)/OX-44(CD37)⁺ CELLS = MOSTLY HEPATIC PRECURSORS, SOME RESIDUAL HEMOPOEITIC
CELL CONTAMINANTS, STROMAL CELLS

OC.3⁻, GRANULAR CELLS = COMMITTED BILE DUCT PRECURSORS (AFP⁺, ALB⁻)

OC.3⁻, GRANULAR CELLS = COMMITTED HEPATOCYTE PRECURSORS (AFP⁺, ALB⁺⁺⁺)

OC.3⁺, AGRANULAR CELLS = EARLY HEPATOBLASTS (AFP⁺⁺⁺, ALBUMIN⁺ AND CK 19⁻)

FIG. 6

5/11

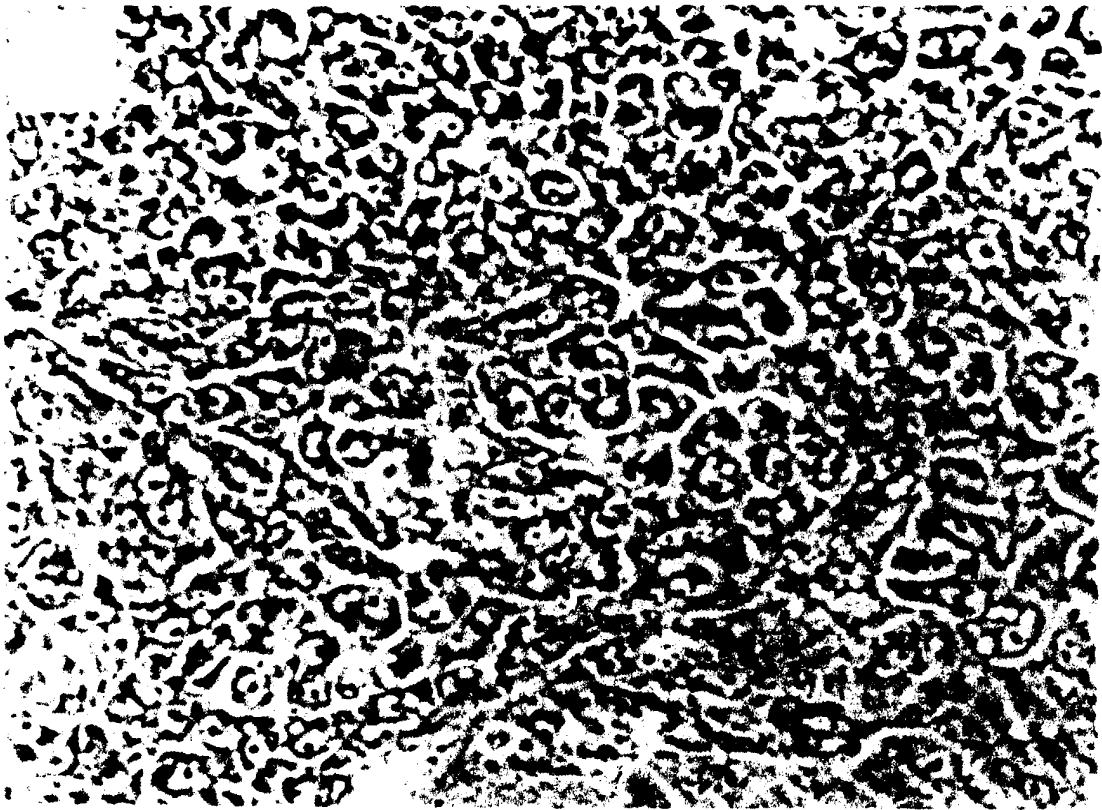
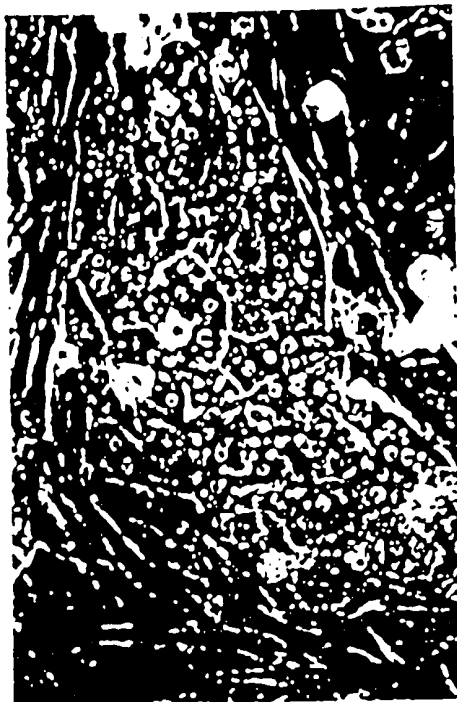


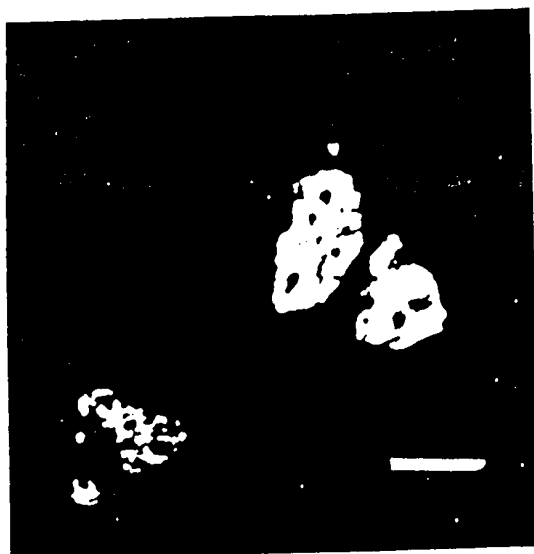
FIG. 5A

Figure 10



SCANNED, # 2

Figure 11



SCANNED # 20

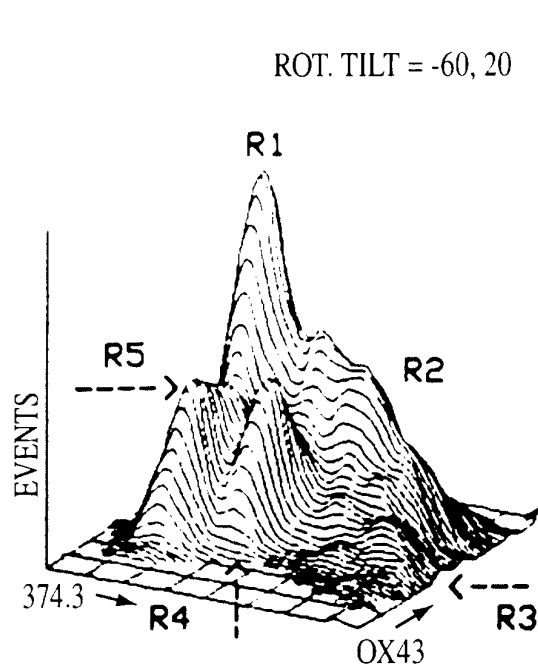


FIG. 1A

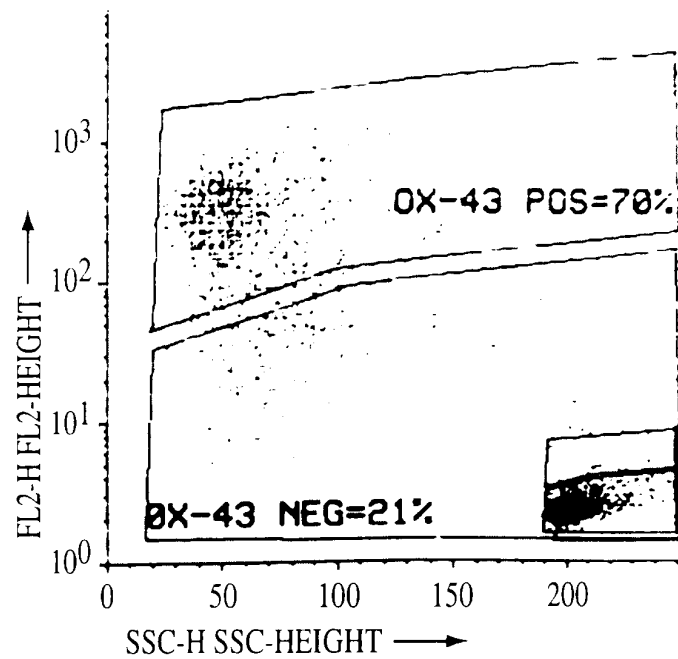


FIG. 1B

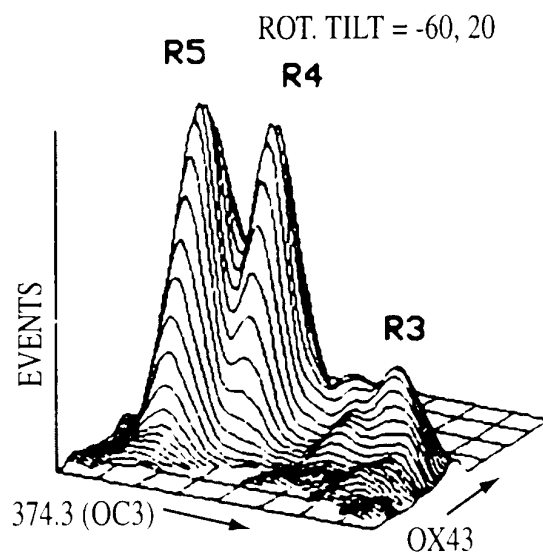


FIG. 1C